

pVITRO2-hygro-mcs

A multigenic plasmid for high levels of expression

Catalog code: pvitro2-mcs

<https://www.invivogen.com/pvitro2-mcs>

For research use only

Version 19A21-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO2-hygro-mcs provided as lyophilized DNA
- 1 ml Hygromycin B Gold at 100 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store Hygromycin B Gold at 4°C or -20°C. The expiry date is specified on the product label.

Quality control

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pVITRO is a family of plasmids developed mainly for in vitro studies. They allow the ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells and the genes of interest are expressed at high levels. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes.

pVITRO2-hygro-mcs plasmid is selectable with hygromycin B in both *E. coli* and mammalian cells. It contains two multiple cloning sites (MCS) for the convenient cloning of two cDNAs.

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning:

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

Hygromycin B usage:

This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

PLASMID FEATURES

• **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein¹. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.

• **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range². The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells.

• **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer³.

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell *et al.*⁴

• **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression⁵.

• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.

• **hph gene** confers resistance to Hygromycin B both in *E. coli* and mammalian cells. In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* is transcribed from the CAG promoter as a polycistronic mRNA and translated via the FMDV IRES.

• **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

• **MCS1 and MCS2:** Each multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

MCS1 contains the following restriction sites:

Age I, Eco RV, Bam HI, Sal I and Avr II

- Age I is compatible with Bsp EI and Sgr AI.
- Eco RV (blunt-end restriction enzyme).
- Bam HI is compatible with Bgl II, Bst YI and Bcl I.
- Sal I is compatible with Ava I and Xho I.
- Avr II is compatible with Xba I, Spe I and Nhe I.

MCS2 contains the following restriction sites:

Sgr AI, Bgl II, Xho I and Nhe I

- *Sgr AI* is compatible with *Bsp EI* and *Age I*.
- *Bgl II* is compatible with *Bam HI*, *Bst YI* and *Bcl I*.
- *Xho I* is compatible with *Ava I* and *Sal I*.
- *Nhe I* is compatible with *Xba I*, *Spe I* and *Avr II*.

1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 91:217-23. 2. Moreau P. *et al.*, 1981. The SV40 72 base repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. *Nucleic Acids Res.* 9:6047-68. 3. Boshart M. *et al.* 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 141:521-30. 4. Carswell S., and Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-58. 5. Ramesh N. *et al.* 1996. High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. *Nucleic Acids Res.* 24(14):2697-700.

TECHNICAL SUPPORT

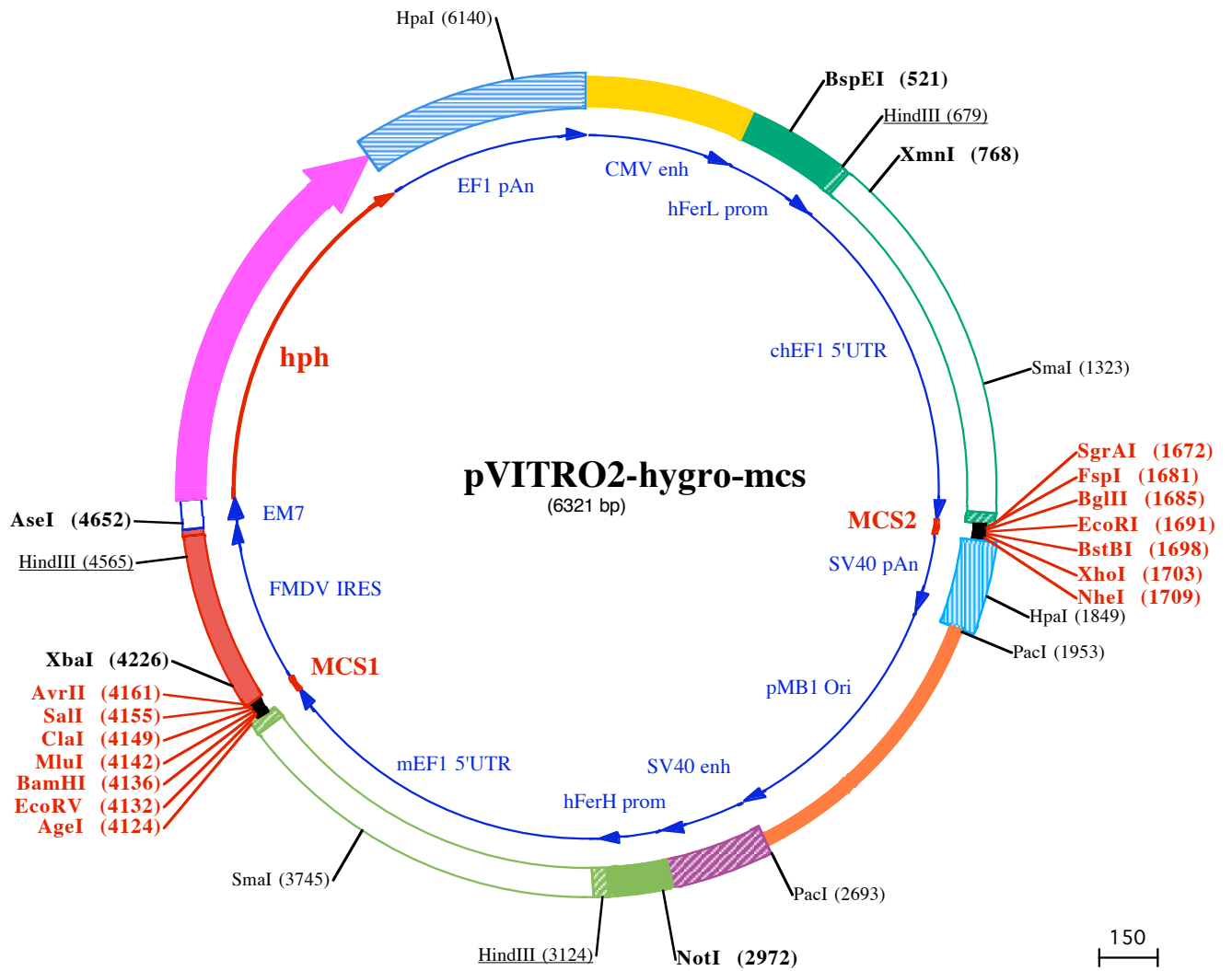
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1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
101 CGCCAATAGGACTTTCCATTGACGTCAATGGTGGAGTATTTACGGTAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
201 TATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
301 GCTATTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGGATTCCAAGTCTCCACCCATTGACGTCAATG
401 GGAGTTTGTGGTACTAGT CAGGGCCCAACCCCCCAAGCCCATTTCAACACGCTGGCGCTACAGGCGCTGACTTCCCTTGTCTTGGGGCGGG
501 GGGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTCGGCCCGCTCTGCCACCGCAGATTGGCCGCTAGGCCCTCCCGAGCGCCTGCC
601 TCCGAGGCGCGGCACCATAAAAGAAGCCGCTAGCCACGTCCCTCGCAGTTCCGGGTCGCCGGTCTGTCTCAAGCTTGGCCGAGAACACAGG
701 taagtgccgtgtgtggttcccgcgggctggcctctttacgggttatggccttgcgtgccttgaattacttccatgccctggctgcagtacgtgattc
801 ttgatcccgagcttcgggttggagtggtgggagagttcgaggcctgcgcttaaggagcccttcgctcgtgcttgagttgaggcctggcttgggagc
901 ctggggccgcccgtgctaactcgtggcacttcgcgctgtctcgtgctttcgttaagtccttagccatttaaaatgggataaccagctgcgagc
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1301 tcaaaatggaggacgcggcgccgggagagcgggagggtgagtcaccacacaaaaggaaaaggcctttcttccatccgtcgttcatgtgactcca
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1501 ccacactgagtggtggagactgaagagttaggccagctggcacttgatgtaattctccttgaatttgcctttttaggttggatcttgcctcattc
1601 tcaagcctcagacagtggttcaaagtttttcttccatttcagGTGTCGTGAAACTACCCCTAAAAGCCACCGGCGTGCACAAGATCTGAATCTTCG
1701 AACTCGAGGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG
1801 TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTATGTTTCAGGTTACAGGGGAGGTGTGG
1901 GAGGTTTTTAAAGCAAGTAAACCTCTACAAATGGTATGGAAATGTTAATTAAGTACCATGACCAAAATCCCTAACGTGAGTTTTCGTTCCTACTG
2001 AGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGGTAATCTGCTGCTTGCAAAACAAAAAACCACCGCTACCAGCG
2101 GTGGTTTGTTCGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACAGCAGAGCGCAGATACCAAACTGTTCTTCTAGTGTAGCCGT
2201 AGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTGCTGTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTGTGTCT
2301 TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGGAGCGAACGACCTAC
2401 ACCGAAGTGAATACCTACAGCTGAGCTATGAGAAAAGCGCCACGCTTCCGAAGGGAGAAAAGCGGACAGGTATCCGTAAGCGGCAGGGTCGGAACAG
2501 GAGAGCGCAGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTGCATTTTTGTGATGCTC
2601 GTCAGGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCGGCCTTTTACGGTCTCTGGCCTTTTGTGTCCTTTTGTCCATGTTCTTAATTAACCTG
2701 CAGGGCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGCAGTTAGGGTGTGAAAGTC
2801 CCCAGGCTCCCAGCAGGAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGAGAAGTATG
2901 CAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTCGCCAGAGCGCGAGGGCTCCAGCGCCGCCCTCCCCACAGCAGGGGGCGG
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3101 GCAGGGCCAGACGTTCTTCCGCGAAGCTTGGCGTACAACGCAggtgagggcggggtgtgcttccgccccgagctggaggtcctgctccgagcg
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3601 caggctggggtagcgtgcccagggccatgtggcccagacccggcacgatctggccttggcggcgccgcttgccctgcctccctaactagggtaggcc

SmaI (3745)

3701 atcccgtccggcaccagttgctgctggaaagatggccgctcccgggcccgttgaaggagctcaaaatggaggacgcccagccggtggagcggg
3801 gggtagtaccacacaaaggaagaggcctggtccctcaccggctgctgcttctgtgacccgctggctctatcggcggcaatagtcacctcgggctt
3901 ttgagcacggtagtcgcccggggggggggatgtaatggcgttggagtttggttcacatttgggtgggtagactagtcaggccagcctggcgtggaa
4001 gtcatttttgaatttgcctctgagttttagcggagctaattctcgggcttcttagcggttcaaaggtatcttttaaaccttttttagGTGTTGTG

EcoRV (4132) MluI (4142) SalI (4155)
AgeI (4124) BamHI (4136) ClaI (4149) AvrII (4161)

4101 AAAACCACCGCTAATTCAAAGCAAACCGGTGATATCGGATCCACGCGTATCGATTGTCGACCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACT

XbaI (4226)

4201 TGAAACTCCGCTGGTCTTTCCAGGCTAGAGGGGTAACACTTTGTAAGTGCCTGGCTCCACGCTCGATCCACTGGCGAGTGTAGTAACAGCACTGTT
4301 GCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCGTCATGTGTGAACC
4401 CCAGCACGGCGACTTTACTGCGAAACCACTTTAAAGTGACATTGAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAG

HindIII (4565)

4501 GTAACACGGGCACTCGGATCTGAGAAGGGGACTGGGCTTCTATAAAAGCGCTCGGTTAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTGGC

AseI (4652)

4601 ACCTTTCCTTTGCAATTAAGTACCTATGAATACAAGTACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAG
4701 GAGGGCCACCATGAAGAACTGAAGTACAGCAACTTCTGTTGAGAAGTTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGCTGTCTGAA
MetLysLysP roGluLeuThrAl aThr Ser Val lGluLysPheLeu l l eGluLysPheAspSer Val l SerAspLeuMetGluLeuSer Glu
4801 GGTGAAGAAAGCAGAGCCTTTCTTTTGTGTTGGAGGAGGTTATGTTCTGAGGGTCAATTTCTGTGCTGATGGTTTTTACAAAGACAGATATGTT
31 Gl yGlu uGluSer ArgAl aPheSer PheAspVal l Gl yGlu yArgGlu yTyrVal l LeuArgVal l AsnSer CysAl aAspGlu yPheTyrLysAspArgTyrVal l T
4901 ACAGACACTTTGCCTCTGCTGCTGCAATTCAGAAGTCTGGACATTGGAGAATTTCTGAATCTCTCACCTACTGCATCAGCAGAAGACACAAGG
64 yr ArgHis sPheAl aSer Al aAl aLeuP ro l l eP roGluVal l LeuAsp l l eGlu yGlu uPheSer Glu uSer LeuThr TyrCys l l eSer ArgArgAl aGlu nGlu
5001 AGTCACTCTCCAGGATCTCCCTGAAACTGAGCTGCCAGCTGTTCTGCAACCTGTTGCTGAAGCAATGGATGCCATTGCAGCAGCTGATCTGAGCCAAACC
97 yVal l Thr LeuGlu nAspLeuP roGlu uThr GluLeuP roAl aVal l LeuGlu nP roVal l Al aGlu uAl aMetAspAl a l l eAl aAl aAl aAspLeuSer Glu nThr
5101 TCTGGATTTGGTCTTTTGGTCCCAAGGCATTGGTCAGTACACCCTTGGAGGGATTTCAATTTGTGCCATTGTGATCCTCATGCTATCACTGGCAGA
131 Ser Glu yPheGlu yProPheGlu yProGlu nGlu y l l eGlu yGlu nTyrThr Thr TrpArgAspPhe l l eCysAl a l l eAl aAspP roHis sVal l TyrHis sTrpGlu nT
5201 CTGTGATGGATGACACAGTTTCTGCTTCTGTGCTCAGGCAGTGGATGAACCTCATGCTGTGGGCAGAAAGATTGTCTGAAAGTACAGACACCTGGTCCATGC
164 hr Val l MetAspAspThr Val l Ser Al aSer Val l Al aGlu nAl aLeuAspGlu uLeuMetLeuTrpAl aGlu uAspCysP roGlu uVal l ArgHis sLeuVal l His sAl
5301 TGATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCAATTGACTGGCTGAAGCCATGTTGGAGATTCTCAATATGAGGTTGCC
197 aAspPheGlu ySerAsnAsnVal l LeuThrAspAsnGlu yArg l l eThr Al aVal l l eAspTrpSer Glu uAl aMetPheGlu yAspSer Glu nTyrGlu uVal l Al a
5401 AACATTTTTTTTGGAGACCTTGGCTGGCTTGCATGGAACAACAACAAGATATTTGAAAGAAGACCCAGAAGTGGCTGGTCCCCAGACTGAGAG
231 Asn l l ePhePheTrpArgP roTrpLeuAl aCysMetGlu uGlu nGlu nThrArgTyrPheGlu uArgArgHis sP roGlu uLeuAl aGlu ySerP roArgLeuArgA
5501 CCTACATGCTCAGAATTTGGCCTGGACCAACTGTATCAATCTCTGGTTGATGGAACTTTGATGATGCTGCTTGGGCACAAGGAAGATGTGATGCCATTGT
264 l aTyrMetLeuArg l l eGlu yLeuAspGlu nLeuTyrGlu nSer LeuVal l AspGlu yAsnPheAspAspAl aAl aTrpAl aGlu nGlu yArgCysAspAl a l l eVa
5601 GAGGCTGGTCTGGAAGTGTGGAAGAAGTCTGCTGCTGTTGGACTGATGGATGTTGAAAGTCTGGCTGACTCTGGAAGC
297 l ArgSer Glu yAl aGlu yThr Val l Glu yArgThr Glu n l l eAl aArgArgSer Al aAl aVal l TrpThrAspGlu yCysVal l Glu uVal l LeuAl aAspSer Glu yAsn
5701 AGGAGACCTCCACAAGACCCAGAGCCAAGGAATGAATATTAGCTAGATTATCCCTAATACCTGCCACCCACTTAAATCAGTGGTGAAGAACGGCT
331 ArgArgP roSer ThrArgP roArgAl aLysGlu u•••••
5801 CAGAAGTGTGTTTCAATTTGGCCATTTAAGTTTAGTAGTAAAAAGCTGGTAAATGATAACAATGCATCGTAAAACCTCAGAAGGAAAGGAAATGTTT

5901 TGTGGACCACCTTTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAAATCAGTACTTTTTAATGAAACAACCTTGACCAAAAATTTGT
6001 CACAGAATTTGAGACCCATTAAGTAAATGAGAAACCTGTGTCTTTGGTCAACCCGAGACATTTAGGTGAAAGACATCTAATTTCTGGTTT

HpaI (6140)

6101 TACGAATCTGGAACCTTCTGAAATGTAATCTTGAGTAACTCTGGTGGAGAATAGGTTGTTTTCCCCACATAATTGGAAGGGGAAGGAAT
6201 ATCATTTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAACAGGCCAAAACTGAGTC
6301 CTTGGTTGCATAGAAAGCTG